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NEW ANTIGEN FOR REACTION OF COMPLEMENT FIXATION IN DIAGNOSIS OF BRUCELLOSIS BY SOVIET SCIENTISTS

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To an increasing extent, the reaction of complement fixation (RSKO) is used by veterinary specialists in the examination of agricultural animals for brucellosis and constitutes a reliable method for its diagnosis.

To receive a clear indication by this reaction, it is necessary to use a specific antigen, the full value of which in many cases depends on the method of its preparation. At present, an extract of brucella cultures proserved by phenol is used an an antigen for this reaction to diagnose brucellosis. Preparation of this antigen takes 5-6 months since the titer of the antigen used causes excessive manipulation for setting up the complement fixation reaction. Thus, there is a need to develop an antigen which will permit more simplified techniques of preparation and utilization. The antigen we have developed answers these requirements.

In the preparation of this antigen, we used the principles of the method tested by Kalinin and Ginzburg, as revealed in their "Modification of the Reaction of Complement Fixation and its Utilization," Medgiz, 1947.

For the preparation of our antigen, which hereafter we will refer to as Antigen No 3, we used a bacterial suspension from types of Brucella bovis, suis, and melitensis. Our Antigen No 3 was tested together with an antigen we called No 2, prepared by extraction of brucella, and an antigen called No 1, prepared by boiling brucella. The reaction of the complement fixation was carried out with blood serum of healthy animals, those suspected of having the disease, and those infected with brucellosis.

The records of these tests are shown in Tables No 1 and 2.

From Table 1 we see that Antigens No 2 and 3, in the reaction of complement fixation, for the most part gave similar results in both the group of healthy animals and in the group of sheep experimentally infected with brucellosis. These same antigens on the group of sheep, whose infection by

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brucellosis was uncertain, confirmed the superiority of the complement fixation reaction over allergy and agglutination reactions, while Antigen No 3 showed a much higher number reacting positively for brucellosis.

The data we received from the blood serum of sheep permitted us to extend the tests to blood serum of healthy cows and to those sick with brucellosis. Blood serum of these animals was tested at the same time by the agglutination reaction and the complement fixation reaction with the three different antigens. The results of this test are arranged in Table 2.

It can be seen from Table No 2 that healthy cows give negative RSK for all three antigens and a negative agglutination reaction for brucellosis. In the herd whose infection by brucellosis was not certain, which was examined by the agglutination reaction, it is noticeable that in four out of six cases where the agglutination reaction was negative, the RSK with Antigen No 3 gave positive results.

In addition, analyses of these results show that in the absolute majority of cases where Antigen No 3 was used, there was more of an indication of the reaction than was received with Antigens No 1 and 2, and in only one case with a positive agglutination reaction was there a negative complement fixation reaction.

The reasons for these discrepancies are a topic for further study.

During the testing of Antigen No 2 in this reaction, it was found that in comparison with the agglutination reaction, No 2 exhibited two cases of positive reactions which the latter had not detected but on the other hand, in four cases of doubtful and positive reactions it gave three negative results and a doubtful one.

Antigen No 1 in the same tests, with a negative agglutination reaction in case, gave a positive result, and a second case a doubtful complement fixation reaction. At the same time, this antigen showed negative RSK in nine cases of positive and two cases of doubtful agglutination reactions.

Table 3 bears out the conclusions made earlier in the study on sheep concerning the superiority of Antigen No 3.

From an analysis of this table it can be seen that Antigen No 3, used in the complement fixation reaction, is first in the number of animals found to have positive RSK, Antigen No 2 is second, and Antigen No 1 is last.

In addition, we tested Antigen No 3 in the complement fixation reaction on 29 test cows suspected of having brucellosis. In the investigation it was shown that one cow, which earlier gave a positive agglutination reaction in our tests gave a positive RSK for brucellosis only with Antigen No 3, Antigen No 1 and 2 giving negative results.

Thus, the tests conducted on 109 sheep and cows for the examination of Antigen No 3 in RSK showed the superiority of this antigen in comparison to Antigens No 1 and 2 in the method of its use, in view of the fact that it does not require titration of the antigen, and by the specificity and clearness of results. The preparation technique is so simple that Antigen No 3 can be used with success at any veterinary bacteriological laboratory.

On the basis of the results of out tests, we consider it possible to recommend Antigen No 3 which we prepared from a mixture of three types of brucella, killed by alcohol and conserved in a one-percent boric acid solution, for practical diagnosis of brucellosis of agricultural animals by the complement fixation reaction method.

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Table 1. Control Tests of Antigens No 2 and No 3 in RSK With Blood Serum of Sheep

Reaction	Groups of Sheep: Experimentally Infected With Brucellosis				Groups of Sheep: Suspected of Disease				Groups of Sheep: Healthy			
	Negative	Doubtful	Positive	Total	Negative	Doubtful	Positive	Total	Negative	Doubtful	Positive	Total
Allergy	-	-	6	6	14	6	10	30	12	-	-	12
Agglutination	-	-	6	6	19	3	8	30	12	-	-	12
Reaction of Complement: Antigen No 2	-	-	6	6	6	1	23	30	12	-	-	12
Reaction of Complement: Antigen No 3	-	-	6	6	3	1	26	30	12	-	-	12

For experimental infection of sheep a dose of 5 billion bacteria of a virulent brucella strain of the type melitensis was used.

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Table 2. Comparative Data of Tests of Antigens No 1, 2, and 3 in Reaction of Complement Fixation With Blood Serum of Cattle

Test No	No of Animals	Reaction of Agglutination — Dilution							Antigen No 1 — Dilution			Antigen No 2 — Dilution			Antigen No 3 — Dilution		
		1/25	1/50	1/100	1/200	1/400	1/800	General Evaluation	1/5	1/10	General Evaluation	1/5	1/10	General Evaluation	1/5	1/10	General Evaluation
								HEALTHY HERD OF CATTLE									
1	47	-	-	-	-	-	-	Negative	-	-	Negative	-	-	Negative	-	-	Negative
2	51	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
3	58	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
4	184	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
5	208	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
6	303	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
7	503	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
8	516	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
9	764	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
10	812	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
								HERD OF CATTLE WITH BRUCELLOSIS									
11	2	++ ++	++ ++	-	-	-	-	Doubtful	-	-	Negative	-	-	Negative	+ +	+	Positive
12	3	++ ++	++ ++	++ ++	+	-	-	Positive	-	+++	Positive	++	+++	Positive	++ ++	++ ++	Positive
13	5	-	-	-	-	-	-	Negative	-	-	Negative	-	-	Negative	++ ++	-	Positive
14	7	++ ++	++ ++	+++	-	-	-	Positive	-	-	Negative	+	+++	Positive	++ ++	++ ++	Positive

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Table 2 (contd)

Test No	No of Animals	Reaction of Agglutination Dilution 1/25	1/50	1/100	1/200	1/400	1/800	General Evaluation	Antigen No 1 Dilution 1/5	1/10	General Evaluation	Antigen No 2 Dilution 1/5	1/10	General Evaluation	Antigen No 3 Dilution 1/5	1/10	General Evaluation
15	9	++	++	+	-	-	-	Positive	-	-	Negative	++	++	Positive	++	++	Positive
16	12	++	++	++	++	-	-	Positive	++	+	Positive	+++	++	Positive	++	+++	Positive
17	13	++	++	+++	+++	+	-	"	-	-	Negative	+	+++	Positive	++	++	"
18	14	++	-	-	-	-	-	Negative	++	++	Positive	++	++	"	++	++	"
19	15	-	-	-	-	-	-	"	-	-	Negative	-	-	Negative	-	-	Negative
20	15/1	++	+++	+	+	-	-	Positive	-	-	"	+	-	Doubtful	++	++	Positive
21	18	++	++	++	++	+	-	"	+++	+++	Positive	++	++	Positive	++	++	"
22	20	++	++	+	-	-	-	"	-	++	"	++	++	"	++	++	"
23	25	++	++	+++	++	-	-	"	+++	++	"	++	++	"	++	++	"
24	27	-	-	-	-	-	-	Negative	-	-	Negative	-	-	Negative	-	-	Negative
25	28	-	-	-	-	-	-	"	-	-	"	-	-	"	-	-	"
26	29	++	++	-	-	-	-	Doubtful	-	-	"	+	-	Doubtful	+++	+	Positive
27	35	++	++	++	++	++	-	Positive	++	-	Doubtful	-	-	Negative	++	++	"
28	37	++	++	+++	++	-	-	"	-	-	Negative	-	-	"	-	-	Negative
29	38	++	+++	+++	+++	+	-	"	-	-	"	++	+++	Positive	++	++	Positive
30	46	++	++	++	++	+	-	"	-	-	"	++	++	"	+++	++	"
31	48	-	-	-	-	-	-	Negative	-	++	Doubtful	+++	++	"	++	+++	"
32	50	++	++	++	+++	+++	+++	Positive	-	++	"	++	++	"	++	++	"

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